Murine Autoimmune Oophoritis, Epididymoorchitis, and Gastritis Induced by Day 3 Thymectomy

Autoantibodies

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In adult mice thymectomized at age 3 days (D3TX), increased incidences and/or levels of organ-specific antibodies to oocytes and/or zona pellucida, to testicular cell-sperm-differentiation antigens (TSDA), and to gastric parietal cells were detected, and these correlated significantly with oophoritis, orchitis (not epididymovasitis), and gastritis, respectively. The autoantibodies occurred in mice with the corresponding endogenous antigens. Thus, anti-oocyte/zona antibodies were detected in female, anti-TSDA antibodies in male, and anti-parietal cell antibodies in both sexes. Anti-oocyte/zona antibodies were first detected at age 5–6 weeks and were absent by 25 weeks. Serum anti-zona antibodies, but not anti-oocyte antibodies, inhibited mouse fertilization *in vitro*. In contrast, antibodies to sperm acrosome and antibodies to sperm surface did not correlate with testicular or epididymal disease. Moreover, both male and female mice had increased levels of anti-sperm surface antibodies, indicating that the sperm antigens detected may not be organ-specific. In addition, sera from 5–10% of D3TX mice reacted with a wide spectrum of epididymal and testicular antigens with defined cellular locations but of yet unknown specificity. Although the incidence of antibodies to cytoskeletal antigens was not significantly elevated after D3TX, anti-nuclear antibodies were more frequently detected in (SWR/J × A/J) F1 (SWRAF1) and (C57 BL/6J × A/J) F1 (B6AF1) mice after D3TX. (Am J Pathol 1987, 126:303–314)

THERE IS a narrow time window in neonatal mice, between Day 2 and Day 4, when thymectomy (D3TX) results in a high prevalence of inflammatory diseases in the ovaries, the testis and the epididymis, the thyroid, the stomach, and the prostate.[1] In addition to serving as an experimental model of autoimmune polyendocrinopathy syndrome in man,[2] autoimmune disease following D3TX provides an approach to studying T-cell subpopulations responsible for induction and prevention of autoimmunity.[3,4] It should also be a useful model for investigating factors that regulate the thymic differentiation of autoreactive T cells against non-major histocompatibility complex antigens.

In the preceding paper,[1] we documented the histopathologic changes in the ovary, the testis, and the stomach of mice after D3TX. The nature and disease correlations of serum autoantibodies in these animals will be described here.

Materials and Methods

Animals and Operations

This study was based on the thymectomized or sham-thymectomized (STX) (C57BL/6 × A/J) F1 (B6AF1), (SWR/J × A/J) F1 (SWRAF1), and BALB/cBy mice described in the preceding paper.[1] Blood was collected with the animals under ether an-

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esthesia from the axillary artery and allowed to clot at 4 C, and serum samples were stored in aliquots at −70 C until study. Outbred Swiss Webster mice were purchased from Charles River Breeding Laboratories (Wilmington, Mass).

Autoantibodies to the Cell Nucleus, Cytoskeleton, Parietal Cells, Sperm Acrosome Antigens, Ovum, Ovaries, Testis, and Epididymis

These antibodies were detected by the standard indirect immunofluorescence (IF) technique.[5] Anti-nuclear Antibodies (ANAs) were detected with frozen sections of mouse kidney fixed in acetone, anti-ovarian antibodies with sections of adult mouse ovaries fixed in 90% ethanol, anti-parietal cell antibodies with unfixed frozen sections of mouse stomach, anti-sperm acrosome antibodies on methanol-fixed smears of caudal epididymal mouse sperm, anti-testis and anti-epididymis antibodies on frozen sections of mouse testis and the entire epididymis fixed in 90% ethanol, and anti-cytoskeleton antibodies (ACSA) on cultured PTK-1 kangaroo rat kidney epithelial cells fixed in 2% paraformaldehyde with 0.05% digitonin (20 minutes at room temperature), or in methanol (−14 C for 5 minutes). To detect surface antigens on mouse oocytes, virgin female mice were given intraperitoneal injections of pregnant mare's serum, and 48 hours later 5 I U of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, Mo). Fifteen hours later, ova from the oviducts were treated with hyaluronidase to disperse the cumulus oophorus, and the zona pellucida was stripped mechanically by pipetting. Mouse sera were studied at a dilution of 1 : 10, and the second antiserum IgG was FITC-conjugated rabbit anti-mouse kappa light chain. The slides were studied as unknowns; and positive results were those with unambiguous positive staining of the respective antigens.

Radioimmunoassay for Antibody to Testis Cell Surface Differentiation Autoantigens (TSDA)[6]

Mouse testes were decapsulated, minced, and incubated in collagenase, 0.5 mg/ml (Type II, Sigma Chemical Co.) and 0.05 mg/ml of deoxyribonuclease (Type I, Sigma Chemical Co.) in phosphate-buffered saline (PBS) that contained 0.1% bovine serum albumin and 0.1% fructose (TSDA buffer). The cells were washed in TSDA buffer and filtered through nylon gauze for removal of tissue fragments. Triplicates of 2.5×10^6 testicular cells (viability exceeded 98%) were incubated with 0.5 ml mouse serum, diluted 1 : 10, 1 : 20, and 1 : 40 in TSDA buffer, for 30 minutes at room temperature. After two washings in the TSDA buffer by centrifugation at 150*g* for 10 minutes, the cells were incubated with 1.5 μg (about 800,000 cpm) of ^{125}I-labeled goat antimouse IgG+IgA+IgM antiserum IgG (Cappel Worthington Biochemicals/Cooper Biomedical, Inc., Malvern, Pa) in 50 μl for 30 minutes at room temperature. After the cells were washed two more times in the TSDA buffer, cell-bound radioactivity was determined in a gamma counter. The results were expressed as percent uptake of the added radioactive antibody at 1 : 20, which fell in the slope of the dose-response curve. Each study also included a standard curve established with a pooled mouse anti-testis antiserum.

Enzyme-Linked Immunoassay (EIA) for Anti-Sperm Surface Antibody

Sperm from the cauda epididymids of B6AF1 mice were washed twice in Tyrode's solution at room temperature. Added to each well of a 96-well microtiter plate precoated with poly-L-lysine 10^5 sperm in 0.1 ml. These were centrifuged at 300*g* for 5 minutes. The adherent sperm were fixed for 2 hours in 1% paraformaldehyde. After the fixative was aspirated, 150 μl PBS containing 1% bovine serum albumin was added, evaporated to dryness, stored at 4 C, and used within 2 weeks. For assaying for antibody, the sperm was rehydrated and the wells were washed twice in PBS. Half a milliliter of mouse serum, diluted 1 : 10, 1 : 20, and 1 : 40 in PBS with 10% goat serum (EIA buffer), each in duplicate, was added to the microtiter well and incubated for 2 hours at room temperature. The wells were then washed five times in EIA buffer and incubated with an appropriate concentration of goat anti-mouse IgG+IgA+IgM antiserum IgG, conjugated with peroxidase, for 2 hours at room temperature. After the wells were washed five more times in the EIA buffer, 50 μl of O-phenylenediamine at 40 mg/dl and 50 μl of hydrogen peroxide (0.04%) were added to each well. Ten to 15 minutes later, 50 μl of 2.5 N H_2SO_4 was added to each well, and the absorbance at 490 nm was determined with an automatic EIA spectrometer. The antibody level was expressed as the optical density of the absorbance extrapolated to the serum dilution of 1 : 10.

Absorption of Antiserum With Tissue Antigens

Serum samples diluted 1 : 5 in PBS were incubated with homogenates of ovary, testis, stomach, kidney, liver, or dissociated testicular cells at 37 C for 60 minutes and overnight at 4 C, then centrifuged at 10,000 rpm. The absorption was repeated twice.

Mouse Fertilization *in Vitro*

The medium used in these studies was modified Krebs-Ringer bicarbonate buffer (IVF buffer).[7] Sperm were collected from the cauda epididymidis of adult Swiss Webster mice, suspended into 0.4 ml of IVF buffer under mineral oil, and incubated for 1 hour at 37 C in 5% CO_2 and air for sperm capacitation to occur. A small volume of sperm was transferred with a calibrated fine glass pipette to 0.2 ml of IVF buffer containing antisperm antiserum or control mouse serum. The final sperm concentration was 1×10^5/ml. Fifteen minutes later, sperm viability, motility, and agglutination were determined under an inverted microscope. The tubal ova that were obtained from hormone-treated virgin Swiss Webster female mice, as described above, were released into the medium by puncturing the ampulla and were mixed with the sperm. After the sperm/ovum mixture was incubated at 37 C in 5% CO_2 and air for 5 hours, the ova were mounted with slight compression between a slide and coverslip, fixed with 2.5% glutaraldehyde and neutral formalin, and stained with 0.25% lacmoid in 45% acetic acid. Evidence of fertilization was the presence of sperm tails and/or male pronuclei in the ova. When serum was studied for anti-ovum antibodies, the cumulus was first removed from the ova by treatment with 0.1% hyaluronidase for about 3 minutes, washed in IVF buffer, and incubated with the immune or control mouse serum.

Statistical Analysis

The Chi-square analysis was used for statistical analysis in all the studies except in anti-sperm surface antibody detection, which was studied by the analysis of variance.

Results

Anti-Ovarian Antibodies

Eighty-two percent of B6AF1 and 97% of SWRAF1 female mice, between 5 and 19 weeks after D3TX, had serum antibodies to oocyte/zona antigens (Table 1). In contrast, anti-oocyte/zona antibodies were not detected after STX. Onset of serum anti-oocyte/zona antibodies coincided with onset of ovarian disease, both at age 5-6 weeks. Inasmuch as the incidence of ovarian diseases in these mice was also about 90%, an excellent correlation existed between oophoritis and anti-oocyte/zona antibodies. However, anti-oocyte/zona antibody response was monophasic; thus, in SWRAF1 females, antibodies were not detected 25 weeks after D3TX. Likewise, antibodies were not detected in 55-week-old BALB/cBy females that had D3TX. The diseased ovaries of these antibody-negative mice were atrophic, without active oophoritis. Male mice did not develop anti-oocyte/zona antibodies after D3TX (Table 1).

Most of the antibodies reacted with the ooplasm, with or without reaction with the zona pellucida, whereas occasional mice (1 in 30) produced antibody to the zona pellucida only (Figures 1 and 2A). Of the 30 antibodies against ooplasm detected in SWRAF1 mice, 17 (56%) reacted only with large oocytes with antral follicles, 1 (3%) reacted with small oocytes only, and 12 (40%) reacted with both large and small oocytes (Figure 1). Thus, the large and the small oocytes have unique cytoplasmic antigens. Anti-oocyte antibodies appeared to react only with cytoplasmic antigens, and they did not bind to the surface of zona-free ova by indirect immunofluorescence. Anti-oocyte and anti-zona antibodies were organ-specific; thus they were removed by absorption with mouse ovaries,

Table 1 — Incidence of Anti-Oocyte/Zona Antibodies and Ovarian Diseases in Mice After D3TX, D7TX, and STX

Strain	Sex	Treatment	Time (weeks)	Disease* (%)	Antibody† (%)
B6AF1	F	D3TX	12-13	27/28 (96)	23/28 (82)
	F	STX	12-13	0/25	0/25
	M	D3TX	7-18	NA	0/24
	M	STX	7-20	NA	0/12
SWRAF1	F	D3TX	5-19	26/31 (84)	30/31 (97)
	F	D3TX	25	10/10 (100)	0/10
	F	STX	5-14	0/17	0/17
	M	D3TX	7-18	NA	0/45
	M	STX	7-20	NA	0/25
BALB/cBy	F	D3TX	55	15/43 (30)	0/43
	F	D7TX	55	0/24	0/24
	M	D3TX	55	NA	0/22
	M	D7TX	55	NA	0/27

*Oophoritis or ovarian atrophy (see Tung et al.[1]).
†Fluorescent anti-ovarian antibody at serum dilution 1:10.
NA, not applicable.

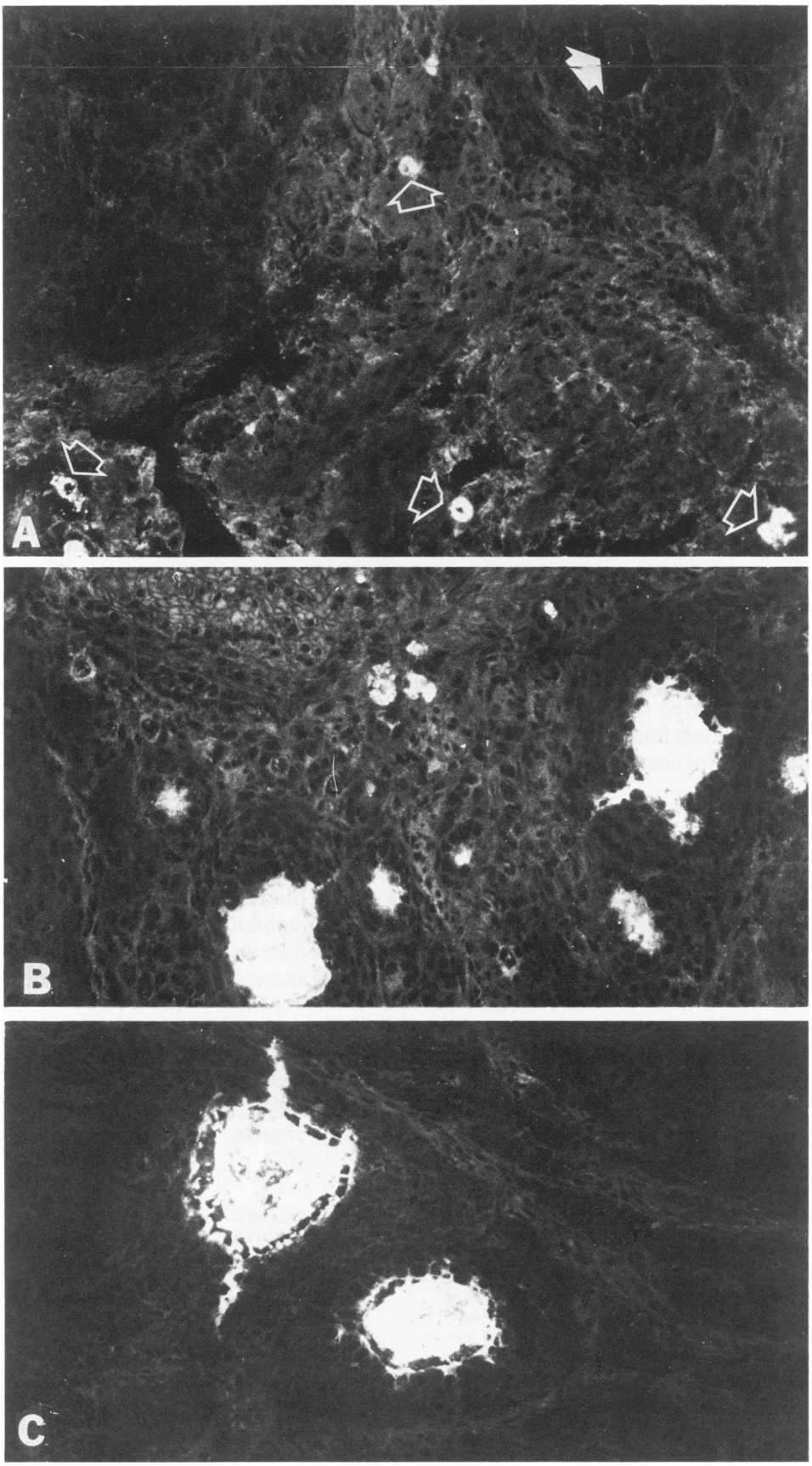

Figure 1 — Anti-oocyte antibodies detected by indirect IF in female mice after D3TX may react with only immature small oocytes (**A**), *hollow arrows* point to positive small oocytes, *solid arrow* points to negative large oocyte), both small and large oocytes (**B**), or only large oocytes (**C**). (×250)

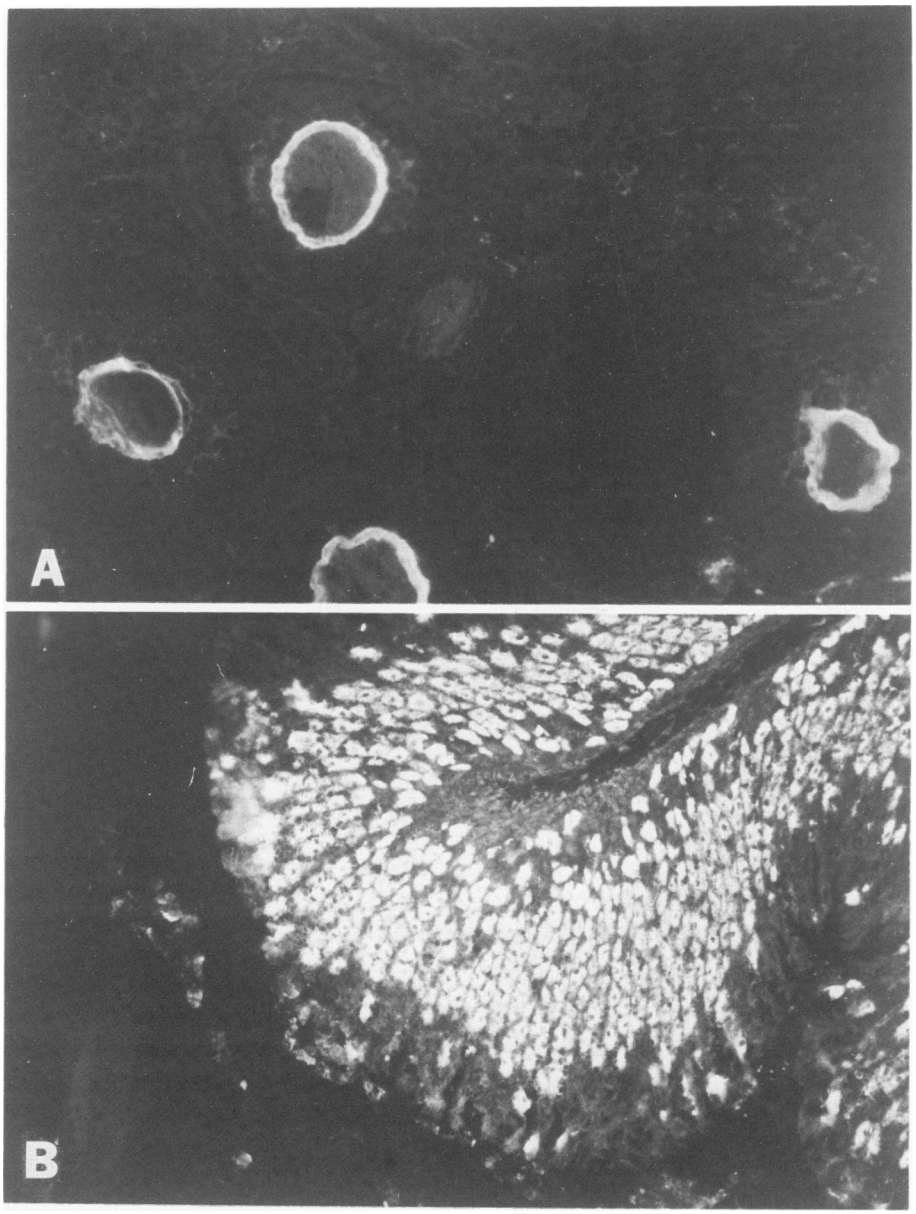

Figure 2—Antibody to zona pellucida in female mice (**A**) and antibody to gastric parietal cells (**B**) after D3TX are detected by indirect IF. (×150)

and not with testes, kidneys, or liver. Although many serum samples reacted with cell nuclei or smooth muscles in the ovarian sections, antibodies to ovary-specific cellular antigens other than oocyte antigens were not detected.

Pooled serum with anti-zona antibodies was found to block fertilization *in vitro* at a dilution of 1 : 10. In contrast, fertilization was not affected by serum with anti-oocyte antibody (Table 2).

Anti-TSDA Antibodies

Significantly higher levels of anti-TSDA antibodies were detected in B6AF1 mice after D3TX than after STX. Moreover, a positive and significant correlation was found between the frequency and levels of anti-TSDA antibody and occurrence of orchitis (Table 3). In contrast, there was no correlation between the anti-TSDA antibody level and epididymitis. Elevations of anti-TSDA antibodies were not found in female mice after D3TX, even though the antibody levels of female mice were higher than those of male mice after STX (Table 4). Anti-TSDA antibodies in the sera of both male and female mice were absorbed by dissociated mouse testicular cells, but not by ovarian, kidney, or liver homogenates, which indicates that TSDA was testis-specific. Serum with high titers of anti-TSDA antibodies did not affect fertilization *in vitro* (data not shown).

Table 2—Effects of Anti-zona and Anti-oocyte Antibodies in Sera of D3TX Females on Murine Fertilization *in vitro*

Experiment	Antiserum	Fertilization rate* at serum dilution of 0†	1:90	1:30	1:10
1	Anti-zona	26/39 (67)	16/30 (53)	28/41 (68)	5/48 (10)
2	Anti-zona	10/19 (53)	36/51 (71)	22/46 (48)	2/29 (7)
3	Anti-oocyte	83/91 (91)	53/54 (98)	53/58 (91)	48/52 (92)
4	Anti-oocyte	NS	23/43 (54)	19/24 (67)	20/30 (67)
5	Anti-oocyte	NS	20/27 (74)	16/23 (70)	18/26 (69)
6	Normal	45/60 (75)	34/44 (77)	25/32 (78)	4/6 (67)
7	CFA	75/88 (84)	80/97 (83)	75/89 (84)	83/106 (79)

*Number of ova fertilized/total ova studies (%). Evidence of fertilization included swollen sperm head and sperm tails inside ooplasm.
†No serum added.
CFA, complete Freund's adjuvant.

Table 3—Relation Between Anti-TSDA Antibodies and Epididymitis or Orchitis in Male B6AF1 Mice After D3TX

Pathology	Number	Antibody incidence (%)	*P*	Antibody level*	*P*
Orchitis	9	7 (78)		16.85 (4.0)	
No orchitis	47	13 (28)	0.01	6.30 (0.3)	0.0002
Epididymitis	26	11 (42)		9.29 (1.6)	
No epididymitis	30	6 (20)	0.15	7.25 (0.9)	0.27

*Percent uptake. Values and the mean and standard error of the mean (in parentheses).

Table 4—Incidence of Anti-TSDA Antibodies in B6AF1 Mice After D3TX, D7TX, and STX

Sex	Time (weeks)	Treatment	Number	Antibody level*	*P*
M	5–20	D3TX	53	8.14 (0.9)	
M	5–20	STX	28	4.69 (0.18)†	0.008
F	5–20	D3TX	32	6.45 (0.63)	
F	5–20	STX	9	6.90 (0.57)†	0.77

*Percent uptake. Values are the mean and standard error of the mean (in parentheses).
†$P = 0.004$.

Anti-Sperm Acrosome Antibodies

InB6AF1 and SWRAF1 male mice, the incidences of anti-acrosome antibodies were comparable after D3TX or STX (Table 5), and there was no correlation between anti-acrosome antibody and orchitis or epididymitis (Table 6). Although the incidence of antibody was higher in 55-week-old male BALB/cBy mice that had D3TX than in those that had D7TX, there was no significant correlation between anti-acrosome antibody and testicular disease (Tables 5 and 6). An increased incidence of anti-acrosomal antibodies was found in male but not female BALB/cBy mice after D3TX (Table 5).

Anti-Sperm Surface Antibodies

Both B6AF1 and SWRAF1 mice had significantly higher levels of anti-sperm surface antibodies following D3TX than STX (Table 7). However, these autoantibodies were elevated after D3TX in both male and female mice of both SWRAF1 and B6AF1 strains. Sera with high levels of anti-sperm surface antibodies had no effect on fertilization *in vitro* (data not shown).

Antibodies to Testicular and Epididymal Antigens After D3TX

Sera from male SWRAF1 mice that had D3TX were found to react with a wide spectrum of antigens in the mouse epididymis and testis as defined by the cellular locations of reaction on indirect immunofluorescence (IF). Two groups of antibodies were detected. Group 1 was antibodies found only in mice after D3TX and not STX, including antibodies to 1) large granular speckles in nuclei of epithelial cells of the caput and corpus epididymis in 2 of 47 (5%) mice (Figure 3A), 2) fine spikes at the luminal surface of epithelial cells in corpus epididymis in 4 of 47 (10%) mice (Figure 3B), 3) ring-shaped antigens surrounding "basal cells" in corpus epididymis in 2 of 47 (5%) mice (Figure 3C), and 4) linear antigens surrounding

Table 5—Incidence of Anti-serpm Acrosome Antibodies in D3TX, D7TX, and STX Mice

Strain	Sex	Treatment	Time (weeks)	Incidence (%)	P
B6AF1	M	D3TX	7–18	8/24 (33)	
	M	STX	7–20	1/12 (8)	0.23
	F	D3TX	12–13	2/15 (13)	
	F	STX	13	2/9 (22)	1.00
SWRAF1	M	D3TX	4–19	16/45 (36)	
	M	STX	5–20	8/25 (32)	0.97
	F	D3TX	4–8	0/18 (0)	
	F	STX	8–19	2/14 (14)	0.36
BALB/cBy	M	D3TX	55	15/17 (88)	
	M	D7TX	55	5/19 (26)	0.0006
	F	D3TX	55	6/21 (29)	
	F	D7TX	55	2/12 (17)	0.73

Table 6—Relation Between Anti-Sperm Acrosome Antibodies and Epididymoorchitis After D3TX or D7TX

Strain	Treatment (weeks)	Antibody*	Epididymitis and/or orchitis	P
B6AF1	D3TX	Positive	4/8	
	(7–18)	Negative	8/16	0.67
SWRAF1	D3TX	Positive	13/16	
	(4–19)	Negative	23/28	0.99
BALB/cBy	D3TX	Positive	10/16	
	(55)	Negative	1/2	0.67
	D7TX	Positive	4/5	
	(55)	Negative	5/14	0.24

*Antibody at serum dilution 1:10.

"Sertoli cell nuclei" (Figure 3D) in 3 of 47 (7%) mice. Finally, an unusual antibody reacted with vascular smooth muscle but not with the muscularis mucosa of the vas deferens and cauda epididymidis (Figure 4A).

Group 2 antibodies were found in both D3TX and STX mice. They included antibodies to 1) cellular interface between adjacent epithelial cells in the corpus epididymis (Figure 4B), 2) brush borders of caput and corpus epididymides (Figure 4C), and 3) discrete granules (? chromatoid bodies) in testicular germinal epithelium (Figure 4D). Although these antibodies were found in comparable prevalence after D3TX or STX, they appeared earlier in mice that had D3TX. The tissue specificity of antibodies to epididymis and testis antigens was not determined because of the limited supply of the serum sample.

With the same serum samples, antibodies to unusual structures in kidneys and ovaries were not detected by indirect IF.

Anti-Parietal Cell Antibodies

BALB/cBy mice showed significantly higher prevalence of anti-parietal cell antibodies after D3TX than D7TX (Table 8). Although anti-parietal cell antibodies were elevated in mice of both sexes after D3TX, the incidence was significantly higher in the female mice. A very good correlation existed between anti-parietal cell antibodies and gastritis (Table 9). Anti-parietal cell antibodies were removed by absorption with gastric homogenate, but not with testis, ovary, kidney, or liver.

ANA and ACSA

ANA was found with higher incidence after D3TX than after STX in both male and female B6AF1 mice and in male SWRAF1 mice (Table 10). In contrast, the incidence of ACSA, including antibodies recognizing components of the microfilaments, microtubules, intermediate filaments, and centromeres, was

Table 7—Effect of D3TX on Antibodies to Sperm Surface Antigens in B6AF1 and SWRAF1 Mice

Mouse strain	Sex	Operation	Number	Antibody level*	P
B6AF1	M & F	STX	27	115 (21)	
		D3TX	69	231 (22)	0.001
SWRAF1	M & F	STX	26	200 (24)	
		D3TX	66	270 (27)	0.01
B6AF1 and SWRAF1	M	STX	33	196 (22)	
		D3TX	130	237 (17)	0.001
B6AF1 and SWRAF1	F	STX	20	92 (17)	
		D3TX	73	240 (20)	0.001

*Anti-sperm surface antibodies expressed as absorbance at 490 nm (mean [SEM]), studied 5–20 weeks after D3TX.

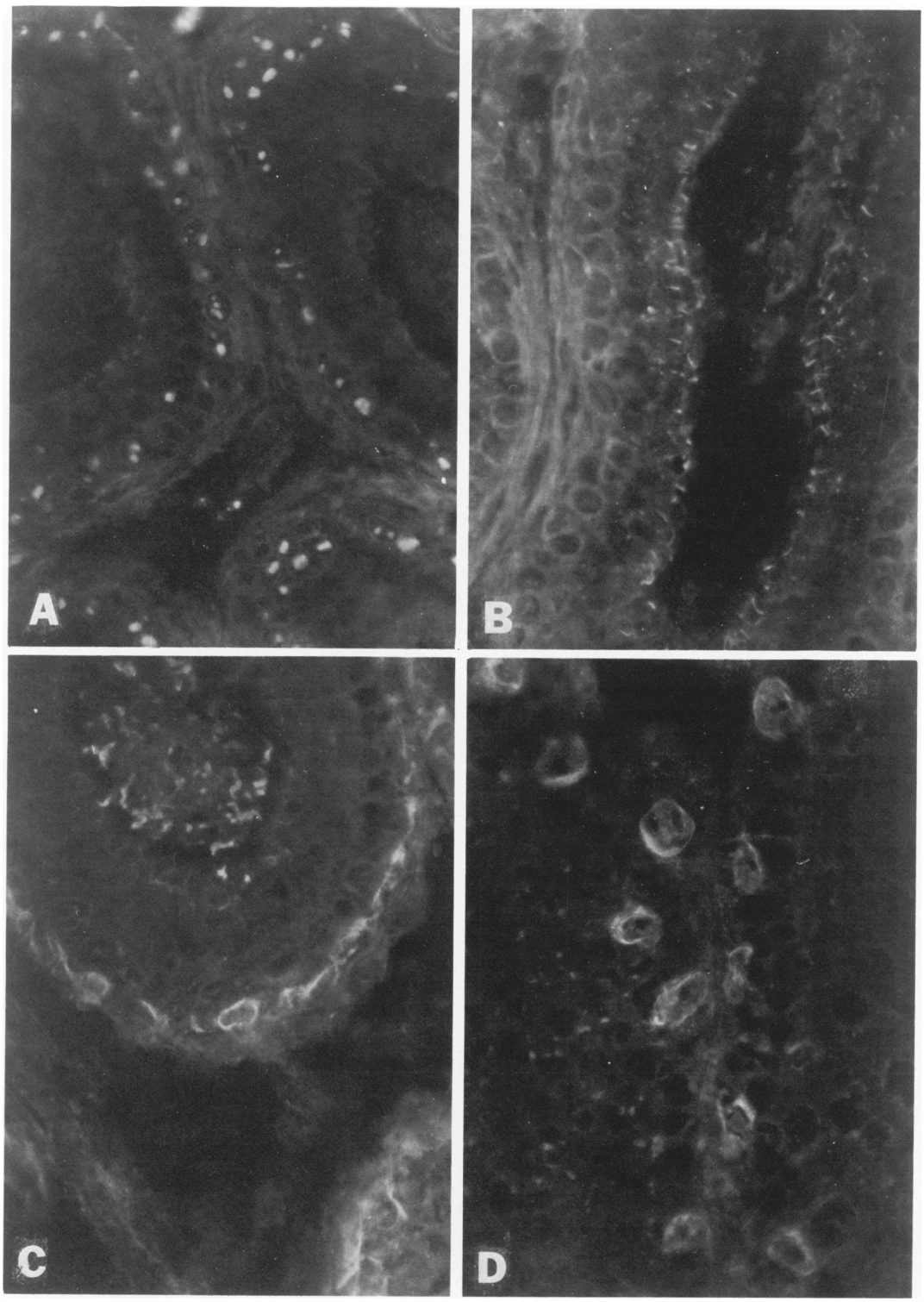

Figure 3 — Antibodies to unusual epididymal and testicular structures are detected by indirect IF in the sera of 5–10% of mice after D3TX, but not after STX. They are: antibodies to coarsely granular nuclear speckles in caput epididymidis (**A**), spikelike structures at the luminal aspects of epithelial cells in corpus epididymidis (**B**), ringlike structures (? basal cells) outside the epithelial cells of corpus epididymidis (**C**), and linear structure surrounding possible Sertoli cell nuclei in testis (**D**). (**A–C**, ×250; **D**, ×800).

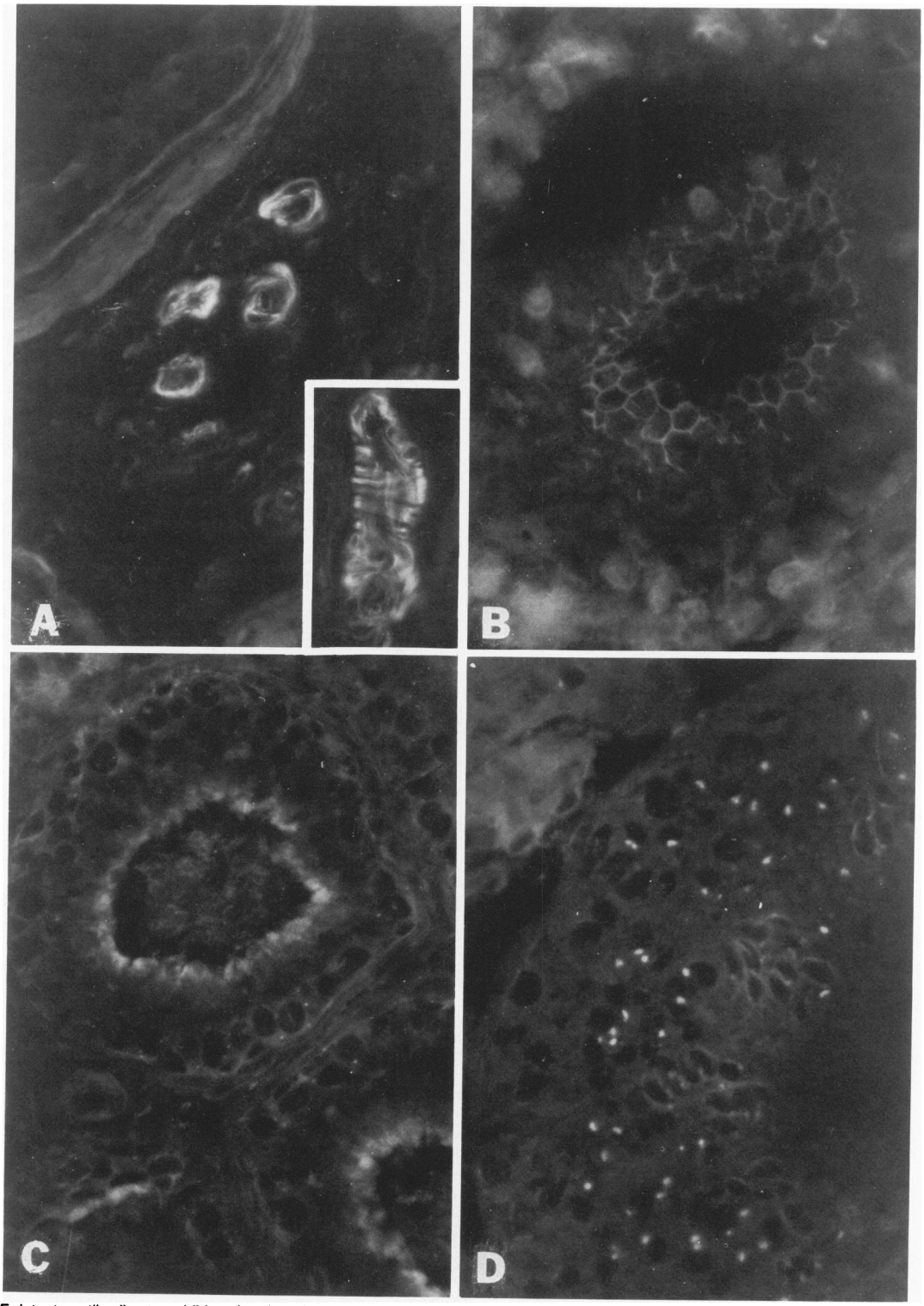

Figure 4—IF detects antibodies to epididymal and testicular structures. Antibody to smooth muscles of small blood vessels, but not muscularis of the vas deferens, is found in one mouse after D3TX (**A**). Other antibodies, found in both D3TX or STX mice, are antibodies to epithelial cellular interface (**B**) and brush border (**C**) of corpus epididymidis and single granules (? chromatid bodies) in germinal epithelium of testis (**E**). (**A**, ×250; **B**–**D**, ×400)

Table 8—Incidence of Anti-Parietal Cell Antibodies in BALB/cBy Mice at 55 Weeks After D3TX and D7TX

Treatment	Sex	Number with antibody* (%) Total number	P
D3TX	M and F	44/88 (50)	
D7TX	M and F	5/51 (10)	<0.00001
D3TX	F	36/66 (55)	
D7TX	F	3/24 (12)	<0.001.
D3TX	M	8/22 (36)	
D7TX	M	2/27(7)	0.03

*Antibodies at serum dilution of 1:10.

Table 9—Correlation Between Anti-Parietal Cell Antibodies and Gastritis in the BALB/cBy Mice 55 Weeks After D3TX

Sex	Pathology	Number with antibody (%) Total number	P
M and F	Gastritis	35/44 (80)	
	No gastritis	2/44 (5)	<0.00001
F	Gastritis	29/36 (81)	
	No gastritis	2/30 (6)	<0.00001
M	Gastritis	5/8 (63)	
	No gastritis	0/14 (0)	<0.004

not statistically different in mice after D3TX and STX.

Discussion

Thymectomy on Day 3 of age resulted in oophoritis and epididymoorchitis in adult B6AF1, SWRAF1, and BALB/cBy mice, and gastritis in adult BALB/cBy mice.[1] In this study, three types of autoantibodies in the sera of these mice were found to correlate with occurrence of disease. Antibodies to oocyte/zona, TSDA, and parietal cells were found in significantly higher incidence and/or levels in mice with oophoritis, orchitis, and gastritis, respectively. Moreover, this study has clearly demonstrated concordance between positive antibody responses and the presence of autoantigens in the host. Thus anti-oocyte/zona antibodies were found exclusively in the females, elevated anti-TSDA antibody levels occurred only in the males, and anti-parietal cell antibodies in both sexes. Although a possible pathogenetic role of anti-TSDA antibody is supported by immunopathologic findings of immune complexes in the testis,[1] further work is required to determine whether any of these antibodies are the causes or the consequences of the disease process. It would seem, however, that endogenous antigens are required for stimulation of the antibody response. In the case of anti-oocyte/zona antibodies, since the onset of serum antibody coincided with that of oophoritis, the antibody response was not merely a consequence of the ovarian disease.

Table 10—Prevalence of Anti-Nuclear Antibodies (ANA) and Antibodies to Cytoskeletal Proteins (ACSA) in Mice After D3TX, D7TX, and STX

Strain	Sex	Treatment	Weeks	Antibody*	Incidence (%)	P
B6AF1	M	D3TX	7–18	ANA	16/24 (67)	
	M	STX	7–20	ANA	2/12 (17)	0.01
	M	D3TX	12–18	ACSA	8/24 (33)	
	M	STX	12–19	ACSA	0/7 (0)	0.20
SWRAF1	M	D3TX	7–19	ANA	9/34 (26)	
	M	STX	7–20	ANA	1/30 (3)	0.03
	M	D3TX	12–19	ACSA	3/9 (33)	
	M	STX	11–20	ACSA	6/10 (60)	0.50
B6AF1	F	D3TX	12–20	ANA	7/9 (78)	
	F	STX	12–20	ANA	2/10 (20)	0.03
	F	D3TX	12–20	ACSA	7/14 (44)	
	F	STX	12–20	ACSA	2/10 (20)	0.42
SWRAF1	F	D3TX	12–20	ANA	3/26 (12)	
	F	STX	12–20	ANA	0/10 (0)	0.65
	F	D3TX	12–20	ACSA	6/26 (22)	
	F	STX	12–20	ACSA	1/10 (10)	0.68
BALB/cBy	M	D3TX	55	ANA	3/22 (14)	
	M	D7TX	55	ANA	4/27 (15)	0.77
	F	D3TX	55	ANA	7/46 (15)	
	F	D7TX	55	ANA	1/24 (4)	0.33

*ANA at serum dilution 1:10 and ACSA at 1:50.

Our findings on anti-oocyte/zona antibodies differ from those of a previous study[8] in several respects. The earlier study detected only antibodies to large oocytes in female B6AF1 mice after D3TX. Our immunofluorescence study clearly shows that unique cytoplasmic antigens were present in both large and small oocytes. The finding of autoantigens in small oocytes provides the basis for successful adoptive transfer of oophoritis in prepuberal mice,[9] which lack mature oocytes. Unlike data reported previously,[8] reaction between anti-oocyte antibodies and the plasmalemma of oocytes was not detected. Indeed, anti-oocyte antibody of high titer had no effect on *in vitro* fertilization. In contrast, fertilization was significantly reduced in the presence of anti-zona antibody, and the serum dilution at which fertilization was inhibited is comparable to that reported for xenoantiserum to zona pellucida.[10] Finally, we did not detect antibodies to surface antigens of ovarian cells between oocytes.

An important finding that this study emphasizes is the monophasic nature of the anti-oocyte antibody response in mice with oophoritis. As the inflammation in the ovaries subsided, serum anti-oocyte/zona antibody also subsided. Thus, the absence of serum autoantibody does not exclude an autoimmune etiology of disease in the ovary. This finding may be relevant to findings in women with secondary amenorrhea.[11] If this disease should have an immunologic basis, detection of serum autoantibodies to ovaries may not be expected unless they were looked for early in the disease.

The incidence of autoantibodies to sperm/testis antigens detected in male mice after D3TX varied, depending on the method of antibody detection. A significant increase in anti-TSDA antibody levels was detected after D3TX, and it correlated with occurrence of orchitis. Contrary to a previous study,[12] a significantly higher incidence of anti-acrosome antibodies was not found in B6AF1 or SWRAF1 mice after D3TX. Although a higher incidence of anti-acrosome antibodies was detected in BALB/cBy mice after D3TX, positive antibodies did not correlate with testicular disease. On the other hand, although anti-sperm surface antibodies detected by the EIA were elevated after D3TX, they occurred to a similar extent in male and female mice. This finding, as well as the disease correlation of this antibody, strongly argues that antigens detected by the solid-phase anti-sperm surface antibody assay are not sperm-specific. Antibodies may be directed against tissue antigens common to both sexes, or to extrinsic antigenic molecules coating sperm.

By indirect IF, sera from mice after D3TX were found to react with a wide spectrum of epididymal and testicular antigens, but not unusual antigens of kidneys or ovaries. These antibodies do not develop after conventional autoimmunization with sperm and/or testis.[13,14] Although the nature of these antibodies has not yet been defined, the finding indicates that D3TX is a useful approach to generating monoclonal antibodies against unusual molecules in the male gonads for physiologic studies.

The higher incidence of anti-nuclear antibodies in mice after D3TX suggests that the propensity to autoimmunity is not confined to organ-specific antigens. However, unlike organ-specific antibodies, the evidence for increase in incidence of autoantibodies to organ-nonspecific antigens after D3TX is not as firmly established. Although a higher incidence of ACSA was detected in mice after D3TX, the incidence was not significantly different from that in STX mice. Moreover, in the SWRAF1 mice, increased ANA incidence was apparent only in male mice that normally have a low incidence of ANA. It is also possible that increased expression of endogenous murine virus may follow D3TX, and this enhances the production of ANA.[15,16]

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